

## New species of *Diamesa* (Diptera: Chironomidae) from Tibet: conspecific males and females associated with mitochondrial DNA

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### Abstract

Undescribed females representing four morphological types were found in a collection of adult *Diamesa* from about 5000 m altitude in Rongbuk, Tibet. Short DNA sequences of cytochrome oxidase subunit 2 were used to associate two single males in the material with conspecific females. *Diamesa solhoyi* n.sp. and *Diamesa aculeata* n.sp. are described. The complete type material and additional specimens have been deposited in the Insect Collection at the Institute of Zoology, Academia Sinica, Beijing (IZAS). The sequences are deposited in Genbank with accession numbers AM051227–AM051233.

**Key words:** Diptera, Chironomidae, *Diamesa*, new species, Tibet, DNA taxonomy

### Introduction

Taxonomic decisions are frequently based on studies of dead specimens that have been collected in remote or otherwise hard-to-access habitats. When organisms cannot easily be studied *in situ*, association of sexes and life-history stages can be a challenge. Taxonomies are accordingly rich in historical examples of erroneous associations of males, females, and immature stages. Since the advent of the polymerase chain reaction and the expanding use of automated DNA sequencing, taxonomists have a means to bridge morphological gaps between life-history stages and to establish species identity based on sequence similarities. Such procedures have recently become known as ‘bar coding’ (Hebert et al. 2003).

*Diamesa* Meigen is a genus of chironomids associated with cool and harsh environments. Larvae are dominant in the kryon zone of glacier fed streams (Sæther 1968, Steffan 1971, Steffan 1974, Flory & Milner 2000, Lods-Crozet et al. 2001) and adults are active on snow at sometimes very low temperatures (Shilova 1978, Koshima 1984). Many *Diamesa* species were originally described from arctic or high-altitude expeditions, and

because several species tend to co-occur at one site, males and females of different species were frequently misassociated in descriptions and identification work. Actual observation of copulation between pairs of non-sister species of *Diamesa* (E. Willassen unpublished) indicates that not even a pair *in copula* is a fool-proof association of sexes. DNA bar coding thus potentially is a useful supplement to traditional morphological studies in taxonomic work on such 'promiscuous' insects.

The Himalaya probably is a core area of *Diamesa* diversity. Nevertheless, relatively little is known about this group of insects in this huge mountain range. In this paper, I report on some material collected by a joint Sino-Norwegian expedition to the Rongbuk Area of Tibet. Comparison with results from previous taxonomic work in the Great Himalaya and neighbouring ranges (Singh 1958, Tokunaga 1966, Reiss 1968, Kaul 1970, Serra-Tosio 1983, Makarchenko & Bulgakov 1986, Sæther & Willassen 1987, Makarchenko 1989, Makarchenko & Kobayashi 1997) suggests that the material includes at least two new *Diamesa* species. This conclusion is based on studies of the two single males in the samples. Predominance of females in the material may reflect a bias of the trapping methods (water-traps and Malaise traps). It is also possible that the samples were retrieved at the tail end of an emergence curve, because females tend to live much longer than males.

## Materials and methods

In the early phase of the work, DNA was extracted by salt and ethanol precipitation (Sunnucks & Hales 1996) from ground thorax. Remaining parts, except wings, were cleared in 8% KOH before mounting for microscopy. I later developed a protocol (Ekrem & Willassen 2004) that retains the thorax undamaged; legs, wings, and male antennae were dissected in 96% ethanol and mounted in Euparal® on a microscope slide. The remaining body was air dried for about 15 minutes at room temperature before extraction, using the animal-tissue protocol provided with the DNeasy Tissue Extraction Kit® (Qiagen). After overnight lysis, the extraction fluid was transferred to a silica column while the exoskeleton was retained in the Eppendorf tube to be washed with distilled water. Occasionally, the exoskeleton was additionally soaked in KOH (8%) and glacial acetic acid in succession to remove incompletely dissolved body tissues and crystallised extraction buffer. The specimens were finally dehydrated in absolute ethanol and mounted together with the legs and wings in Euparal. Morphological terms and standard abbreviations used in the descriptions are defined by Sæther (1980). Standard measurements are given as ranges when more than one specimen was measured. The drawings were made by the author, initially aided by a camera lucida on a Leitz Dialux microscope, and in the final stage by the software Photoshop. All the types and additional specimens have been deposited in the Insect Collection at the Institute of Zoology, Academia Sinica (IZAS), 19 Zhongguancun Lu, Haidian, Beijing 100080, China.

CoxII replicates were produced with the standard primers (Simon & al. 1994) TL2-J-3034 (aat atg gca gat tag tgc a) and TK-N-3785 *alias* B-tLYS (gtt taa gag acc agt act tg). When the full length of CoxII did not amplify well, C2-J-3400 (att gga cat caa tga tat tga) was used as an alternative to TL2-J-3034. PCRs were run in a volume of 25 µl with 1–2 units AmpliTaq® polymerase (Perkin Elmer). Totally 45 cycles were run with 60 seconds in each thermal phase of a cycle and a final extension phase of seven minutes. Primer annealing temperatures were set to 50–52 °C, but a step-up procedure with initially 10 cycles of annealing at 45–48 °C was sometimes used. Denaturation and extension temperatures were 94 °C and 72 °C, respectively. PCR products were purified using QIAquick PCR Purification Kit® (Qiagen). Purified products were sequenced in both directions, using BigDye® termination reactions (Perkin Elmer), with the same set of primers used for amplification. Reactions were analysed on an ABI 377 sequencer. Sequence files were edited and assembled with Sequencher 3.1.1 (Gibbs & Cockerill 1995). Genetic distances were computed with PAUP\* (Swofford 2002). The sequences are deposited in Genbank with accession numbers AM051227 – AM051233.

### Sequencing results

The full length of CoxII was difficult to retrieve for some of the specimens and comparisons of aligned sequences were limited to 368 positions in the downstream part of the gene. Sequence differences are summarized in Table 1.

**TABLE 1.** Numbers of nucleotide differences (above diagonal) and p-distance (below diagonal) in pairwise comparisons of partial CoxII from *Diamesa* males and females found at Rongbuk, Tibet. Codes refer to DNA extracts and voucher specimens.

	Sex and code	1	2	3	4	5	6	7	Identification
1	♀ EW96	-	3	39	37	37	37	33	<i>D. sp1</i>
2	♀ EW70	0.0082	-	37	36	36	36	31	<i>D. sp1</i>
3	♀ EW216	0.1060	0.1005	-	31	31	31	38	<i>D. sp2</i>
4	♂ EW41	0.1005	0.0978	0.0842	-	0	0	29	<i>D. solhoyi</i> n.sp.
5	♀ EW69	0.1005	0.0978	0.0842	0.0000	-	0	29	<i>D. solhoyi</i> n.sp.
6	♀ EW164	0.1005	0.0978	0.0842	0.0000	0.0000	-	29	<i>D. solhoyi</i> n.sp.
7	♂ EW36	0.0900	0.0842	0.1033	0.0788	0.0788	0.0788	-	<i>D. aculeata</i> n.sp.

It seems fair to conclude that male EW41 can be associated with females EW69 and EW164 because there is no difference among the samples in this part of CoxII. None of the remaining females can be associated with male EW36. Thus, four species are indicated in the material. The nucleotide divergence is about 8–10% between species and 0–0.8% within species.

## Descriptions

### *Diamesa solhoyi* new species

(Figs. 1A–E)

*Type material*: Holotype male labelled: China: Tibet, Rongbuk, 5000 m a.s.l., 10.Jul. 93, leg. T. Solhøy, det. E. Willassen, EW41. Paratypes with same locality as holotype except: 7.Jul.1993, EW69, 1 female; 8–10.7.93, EW164, 1 female; 8–10.7.93, 1 female. All specimens in Academia Sinica, Zoological Institute, Beijing, China.

*Etymology*: This species is named after Torstein Solhøy, University of Bergen, who collected the specimens.

*Genetics*: Genbank accession numbers AM051230–32, mitochondrial COII gene for cytochrome oxidase subunit II.

#### *Male imago* ( $n = 1$ )

Head: Flagellomeres lost. Antennal pedicel large, with 3 setae. Eyes hairy, moderately projecting dorsomesally. Coronal suture complete. Temporal setae weak and transparent, including 18–20 verticals and six postorbitals. Clypeus 152  $\mu$ m long, 143  $\mu$ m wide; with 9 setae. Palps partly lost. Tentorium (Fig. 1A) with conspicuous anterolateral projection.

Thorax damaged in DNA preparation.

Wing: Length 3.98 mm. VR 0.85. Membrane without setae, punctuation of microtrichia distinct at 100x magnification. Costa produced beyond  $R_{4+5}$ . R with 20 setae.  $R_1$  with 14 setae and 2 sensillae campaniformia.  $R_{2+3}$  with 2 sensillae campaniformia.  $R_{4+5}$  with 6 setae and 3 sensillae campaniformia. Alula without setae. Squama with marginal fringe of about 40 setae.

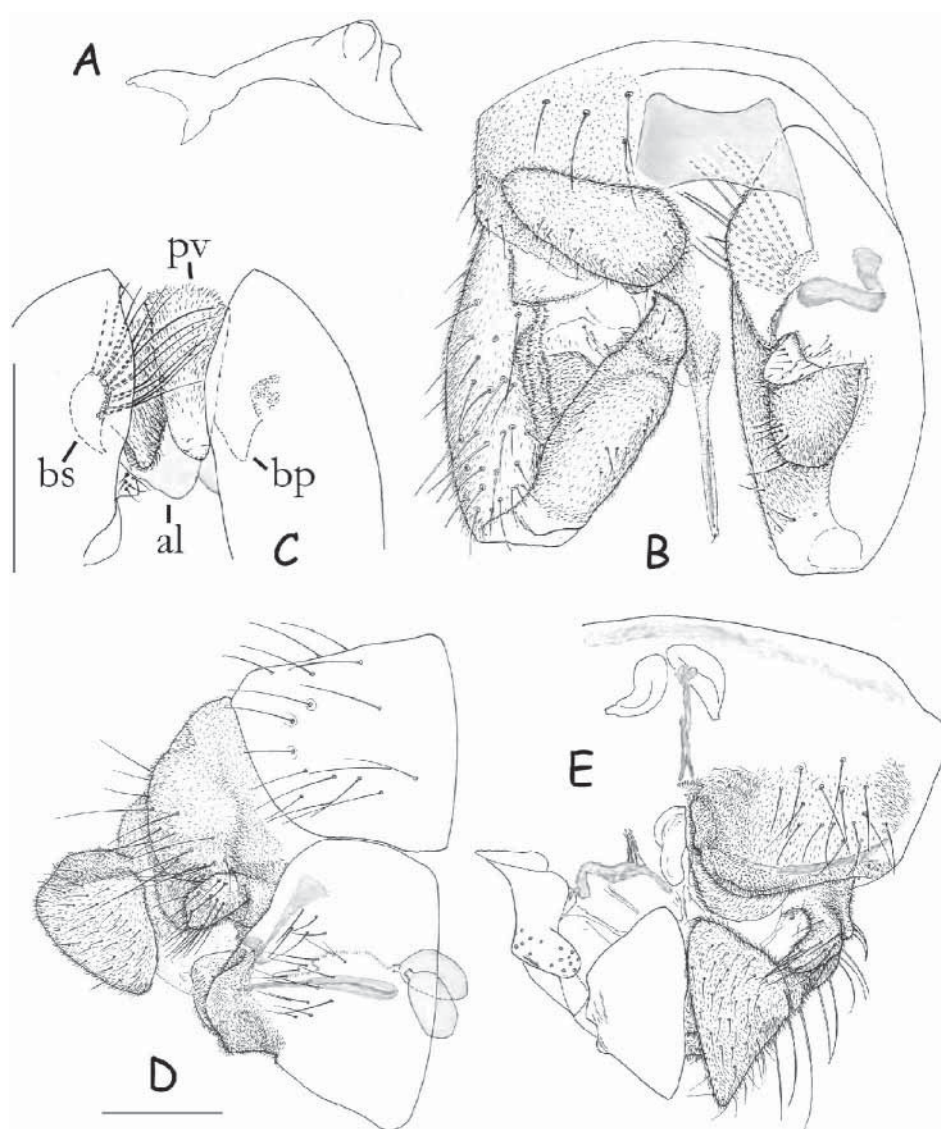
Legs: Comb on  $ti_3$  with 17 setae. Tarsi 1–3 respectively with following numbers of apical/preapical pseudospurs: 2/6, 2/0, 2/0 on  $p_1$ ; 2/13, 2/6, 2/1 on  $p_2$ ; 2/14, 2/6, 2/1 on  $p_3$ . Hind leg with about 45 sensillae chaetica distributed from 0.3 to 0.6 of  $ta_1$ .

**TABLE 2.** Lengths (in  $\mu$ m) and proportions of legs in male *Diamesa solhoyi* new species.

	fe	ti	$ta_1$	$ta_2$	$ta_3$	$ta_4$	$ta_5$	LR	BV	SV
$p_1$	1536	1914	1370	673	407	133	152	0.72	3.53	2.52
$p_2$	1701	1772	945	512	303	123	152	0.53	4.06	3.68
$p_3$	1961	2162	1394	748	417	142	156	0.64	3.77	2.96

Hypopygium (Fig. 1B–C): Sternapodeme trapezoid with concave anterior margin, pigmentation weak. Pars ventralis long and broad, basal  $\frac{3}{4}$  with microtrichia. Aedeagal lobes visible as faint hyaline plates with rounded, weakly pigmented margins. Basal plate of gonocoxite with blunt caudal projection reaching superior volsella; ventromesal surface with long microtrichia. Basimedial setal cluster well developed, slightly directed anteriad; base somewhat prolonged caudally to ledge with marginal setae. Superior volsella

somewhat orthoclad-like, nearly devoid of microtrichia, but with few weak setae dorsally and ventrally. Medial field well delineated; with dense microtrichia, but with setae near ventral margin only. Gonostyli massive with distal constriction, apex acutely pointed with 1 megaseta. Tergite IX divided into 2 protrusions, each with about 20 weak setae. Tergal bands inconspicuous. Anal point inserted below tergal protrusions; base broad and triangular, distal part slender with indication of longitudinal ridge and with setiform sensilla at apex.



**FIGURES 1A–E.** *Diamesa solhoyi* new species. A, male tentorium. B, male genitalia in dorsal view. C, ventral view of pars ventralis (pv), basal plate (bp), basimedial setal cluster (bs) and aedeagal lobe (al). D, female genitalia in lateral view. E, female genitalia in ventral view. Scale bars (C–D) are 250  $\mu$ m.

*Female imago (n = 2)*

Head: Antenna with 7 flagellomeres. AR 0.28–0.39. Scape occasionally with 1 seta. Pedicel with 2 or 3 setae. Dorsal sensilla coeloconica on flagellomeres 1–3. Coronal suture distinct. Number of temporal setae 34–46, including 13–14 postorbitals. Eyes hairy, moderately projecting dorsomesally. Clypeus 143–160 long 166–180 wide, with 12–15 setae. Lengths of palpomeres ( $\mu\text{m}$ ): 47–57, 57–114, 162–180, 152–199, 238–322. Second palpomere with dorsodistal campaniform sensilla. Third palpomere with sensory pit. Tentorium with conspicuous anterolateral projection.

Thorax: Antepronotum with 14–23 lateral setae. Acrostichals absent. Dorsocentrals 14–17 in 1 row except anteriorly, 11–13 prealars behind callus, about 45–50 scutellars. Protuberance of epimeron II with 3–10 setae.

Wing: Length 4.32–4.56 mm. VR 0.86–0.88. Punctuation of membrane distinct at 40x magnification. Costa moderately produced beyond  $R_{4+5}$ . R with 21 or 22 setae.  $R_1$  with 19–26 setae and 2 sensillae campaniformia.  $R_{2+3}$  with 2 or 3 sensillae campaniformia.  $R_{4+5}$  with 18–21 setae and 3 or 4 sensillae campaniformia. Alula with 7–9 setae. Squama with marginal fringe of about 55 setae.

Legs: Comb of hind tibia with 17 setae. Tarsi 1–3 respectively with following numbers of apical/preapical pseudospurs: 2/8–13, 2/0–4, 2/0 on  $p_1$ ; 2/14–16, 2/7, 2/0–2 on  $p_2$ ; 2/20–22, 2/8, 2/4 on  $p_3$ . Hind leg with about 350 or more sensilla chaetica distributed from 0.09–0.1 to 0.70–0.74 on in  $ta_1$ .  $Ta_4$  cordiform.

TABLE 3. Lengths (in  $\mu\text{m}$ ) and proportions of legs in female *Diamesa solhoyi* new species.

	fe	ti	$ta_1$	$ta_2$	$ta_3$	$ta_4$	$ta_5$	LR	BV	SV
$p_1$	1677–1796	2008–2055	1441–1465	644–673	398–407	152–161	152–180	0.71–0.73	3.77–3.92	2.55–2.60
$p_2$	1866–1890	1866–1890	921–933	493–512	294–313	142–161	161–180	0.49–0.50	4.04–4.26	4.03–4.08
$p_3$	2079–2315	2292–2315	1536–1559	853	417–455	161–171	171–189	0.66–0.68	3.61–3.70	2.82–2.89

Genitalia (Fig. 1D–E): Sternite VIII with 14–17 setae on each side, gonocoxapodeme fairly distinct. Gonapophysis VIII with slightly elevated ridge and weak flap that barely covers base of ventrolateral lobe. Seminal capsules with short, sclerotised neck, capsule surface with weak granulation. Seminal ducts relatively short. Gonocoxite IX with thumblike projection curving mesad, 16–19 setae mainly on projection. Tergite IX deeply divided into 2 protrusions, each with about 16 or 17 setae near posterior and lateral margin. Segment X more or less concealed by projection of GcIX, posterolateral protrusion somewhat oval in lateral and triangular in ventral view. Cerci ear-shaped in lateral view.

*Remarks:* Males of *D. solhoyi* may be readily identified by the unique combination of genitalic characters. Although the flagellomeres are lost in the single available male specimen, the size of the pedicel and the conspicuous anterolateral projection of the tentorium (Fig. 1A) indicate that the male antennae are normal sized with 13 flagellomeres.



Females may be recognised by the thumb-shaped gonocoxite IX, which has a roughly trapezoid profile in lateral view.

***Diamesa aculeata* new species**  
(Figs. 2A–E)

Holotype male: China: Tibet, Rongbuk, 5000 m a.s.l., 7.Jul. 1993, leg. T. Solhøy, det. E. Willassen, EW36. In Academia Sinica, Zoological Institute, Beijing.

*Etymology*: The species name is from Latin *aculeatus*, sharp-pointed, referring to the cuspidate microtrichia on the mesal edge of the male gonocoxite.

*Genetics*: Genbank accession number AM051233, mitochondrial COII gene for cytochrome oxidase subunit II.

*Male imago* ( $n = 1$ )

Head: Antenna plumose with 13 flagellomeres; longest antennal seta longer than ultimate flagellomere; AR 1.2; pedicel large, with 3 setae. Eyes moderately projecting dorsomesally; with pubescence between ommatidia. Coronal suture complete. Temporal setae including 3 orbitals, 17 verticals, and 9? postorbitals. Clypeus 130  $\mu\text{m}$  long, 107  $\mu\text{m}$  wide; with 12 setae. Palpomere lengths ( $\mu\text{m}$ ): 37, 85, 133, 126, 211. Tentorium with conspicuous anterolateral projection.

Thorax damaged in DNA preparation.

Wing: Length 3.17 mm. VR 0.88. Membrane without setae, punctuation of microtrichia distinct at 100x magnification. Costa produced beyond  $R_{4+5}$ . R with 15 setae,  $R_1$  with 12 setae and 2 sensillae campaniformia,  $R_{2+3}$  with 2 sensillae campaniformia,  $R_{4+5}$  with 3 setae and 1 sensilla campaniformia. Alula without setae. Squama with marginal fringe of about 35 setae.

Legs: Comb on  $ti_3$  with 15 setae. Tarsi 1–3 respectively with following numbers of apical/preapical pseudospurs: 2/0, 2/0, -/- on  $p_1$ ; 2/7, 2/5, 2/0 on  $p_2$ ; 2/10, 2/5, 2/0 on  $p_3$ . Hind leg with about 25 sensillae chaetica distributed from 0.45 to 0.52 of  $ta_1$ .

**TABLE 4.** Lengths (in  $\mu\text{m}$ ) and proportions of legs in male *Diamesa aculeata* new species.

	fe	ti	$ta_1$	$ta_2$	$ta_3$	$ta_4$	$ta_5$	LR	BV	SV
$p_1$	1229	1536	1087	521	-	-	-	0.71	-	2.54
$p_2$	1252	1370	685	369	208	90	95	0.50	4.34	3.83
$p_3$	1465	1583	1087	568	284	114	123	0.69	3.79	2.80

Hypopygium: Sternapodeme arced. Pars ventralis moderately large (Fig. 2B); with longitudinal striae, but without microtrichia. Aedeagal lobes sickle-shaped, mesal margin well sclerotised. Basal plate of gonocoxite weakly developed. Basimedial setal cluster absent. Medial field almost completely devoid of microtrichia dorsally; dorsal edge of mesal margin (Fig. 2A) with ornamentation of thornlike microtrichia that are particularly

dense on the ventral side (Fig. 2C); ventromesal margin of gonocoxite with setae and weak microtrichia (Fig. 2B) Gonostyli somewhat flattened and twisted, apex with sclerotised point and 1 megaseta. Tergite IX divided into 2 oval protrusions, each with about 15 moderately long setae. Tergal bands relatively distinct, running from anterior margin of tergite IX to the free base of anal point. Anal point inserted between tergal protrusions; base broad with microtrichia; distal part slender and nude, apex slightly expanded with 1 distal peg-sensilla (Fig. 2D).

*Female imago* ( $n = 1$ , putative association)

Head: Antennae partly lost. Pedicel with 5 or 6 setae. Coronal suture complete. Temporal setae more or less contiguous, about 40 on each side, including 9 postorbitals. Eyes pubescent, moderately projecting dorsomesally. Clypeus 167  $\mu\text{m}$  long, 152  $\mu\text{m}$  wide, with 24 setae. Palps lost. Tentorium with conspicuous anterolateral projection.

Thorax: Antepronotum with 9 lateral setae. Acrostichals absent. Dorsocentrals 16 in 1 row, except anteriorly; 9 prealars behind callus, and about setae on 40 scutellum. Protuberance of epimeron II with 8 setae.

Wing: Length 4.11 mm. VR 0.91. Punctuation of membrane visible at 125x magnification. Costa well produced beyond  $R_{4+5}$ . R with 18 setae.  $R_1$  with 19 setae and 2 sensillae campaniformia.  $R_{2+3}$  with 2 sensillae campaniformia.  $R_{4+5}$  with 18 setae and 3 sensillae campaniformia. Alula with 5 setae. Squama with marginal fringe of about 60 setae.

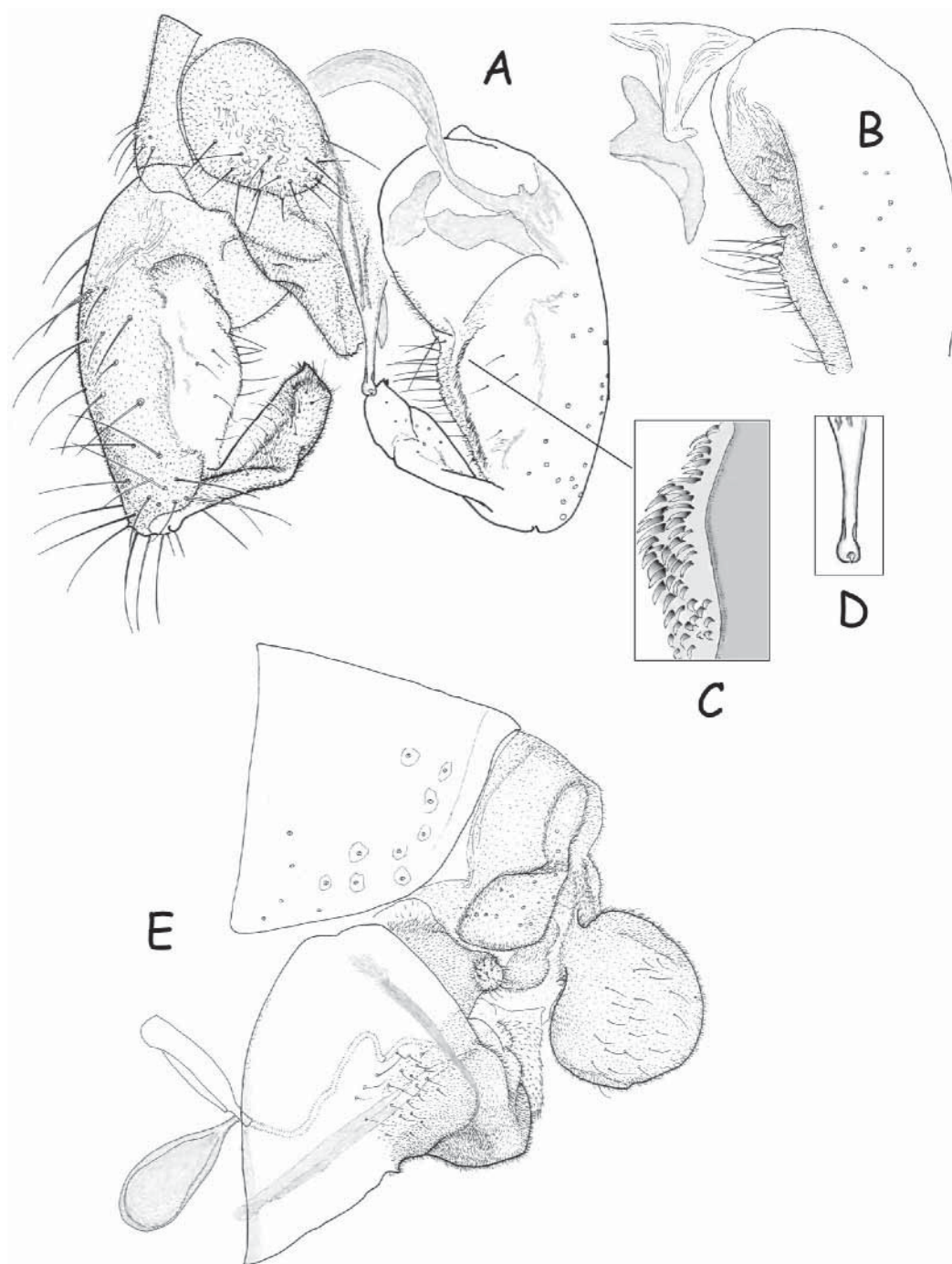
Legs: Lost except right hind femur and tibia. Comb of hind tibia with 15 setae.

Genitalia (Fig. 2E): Sternite VIII with about 20 short setae on each side. Gonocoxapodeme conspicuous with distinct anteromesal curve. Gonapophysis VIII with flap covering mesal part of ventolateral lobe. Seminal capsules elongate with long neck, capsule surface with weak granulation. Seminal ducts with few bends. Gonocoxite IX with laterally directed knoblike projection; about 15 weak setae confined to knob. Tergite IX completely divided into 2 plates, of which broader lateral parts are slightly more sclerotised; each plate with about 20 relatively short setae. Segment X slightly protruding laterad. Cerci rounded in lateral view.

*Remarks:* The male of *D. aculeata* is superficially similar to *Diamesa bertrami* Edwards and also to the Himalayan *Diamesa kasaulica* Pagast described by Serra-Tosio (1983). The ornamentation of the mesal edge and otherwise smooth medial field may serve as diagnostic features of male *D. aculeata*. Both *D. bertrami* and *D. kasaulica* have hairy eyes as opposed to the weakly pubescent eyes in *D. aculeata*. The only female specimen with pubescent eyes in the Rongbuk material presumably also belongs to *D. aculeata*. Antennae, palps, and most of the legs are lacking and the wings were preserved in a folded state. A short, partial Cox II sequence was recovered from the male of *D. aculeata*, but sequences were not obtained from the putative female.

*Additional material:* China: Tibet, Rongbuk, 5000 m a.s.l., 9.Jul. 1993, Malaise Trap, leg. T. Solhøy, det. E. Willassen; 1 female (IZAS).





**FIGURES 2A–E.** *Diamesa aculeata* new species. A, male genitalia in dorsal view. B, ventral view of pars ventralis, aedeagal lobe, basal plate and ventromesal margin of gonocoxite IX. C, ventral view of cuspidate microtrichia along dorsomesal edge of medial field. D, apex of anal point. E, female genitalia in lateral view.

***Diamesa* sp. Himalaya1**  
(Figs. 3A–B)

*Female imago* ( $n = 2$ )

Head: Antenna with 7 flagellomeres. AR 0.50–0.53. Pedicel with 2 setae. Dorsal sensilla coeloconica on flagellomeres 1–3. Coronal suture distinct. Temporal setae weak, 32–36 in number including 11–13 postorbitals, frontals slightly separated from remaining temporals. Eyes hairy, moderately projecting dorsomesally. Clypeus 123–133 long, 123–133 wide, with 11–16 setae. Lengths of palpomeres ( $\mu\text{m}$ ): 38, 76, 109, 118, 241. Second palpomere with distal campaniform sensilla. Third palpomere with sensory pit. Tentorium with conspicuous anterolateral projection.

Thorax: Anteprenotum with 6–9 lateral setae. Acrostichals absent. Dorsocentrals 9–11 in 1 row, 7–9 prealars behind callus, 39–41 scutellars. Protuberance of epimeron II with 4–6 setae.

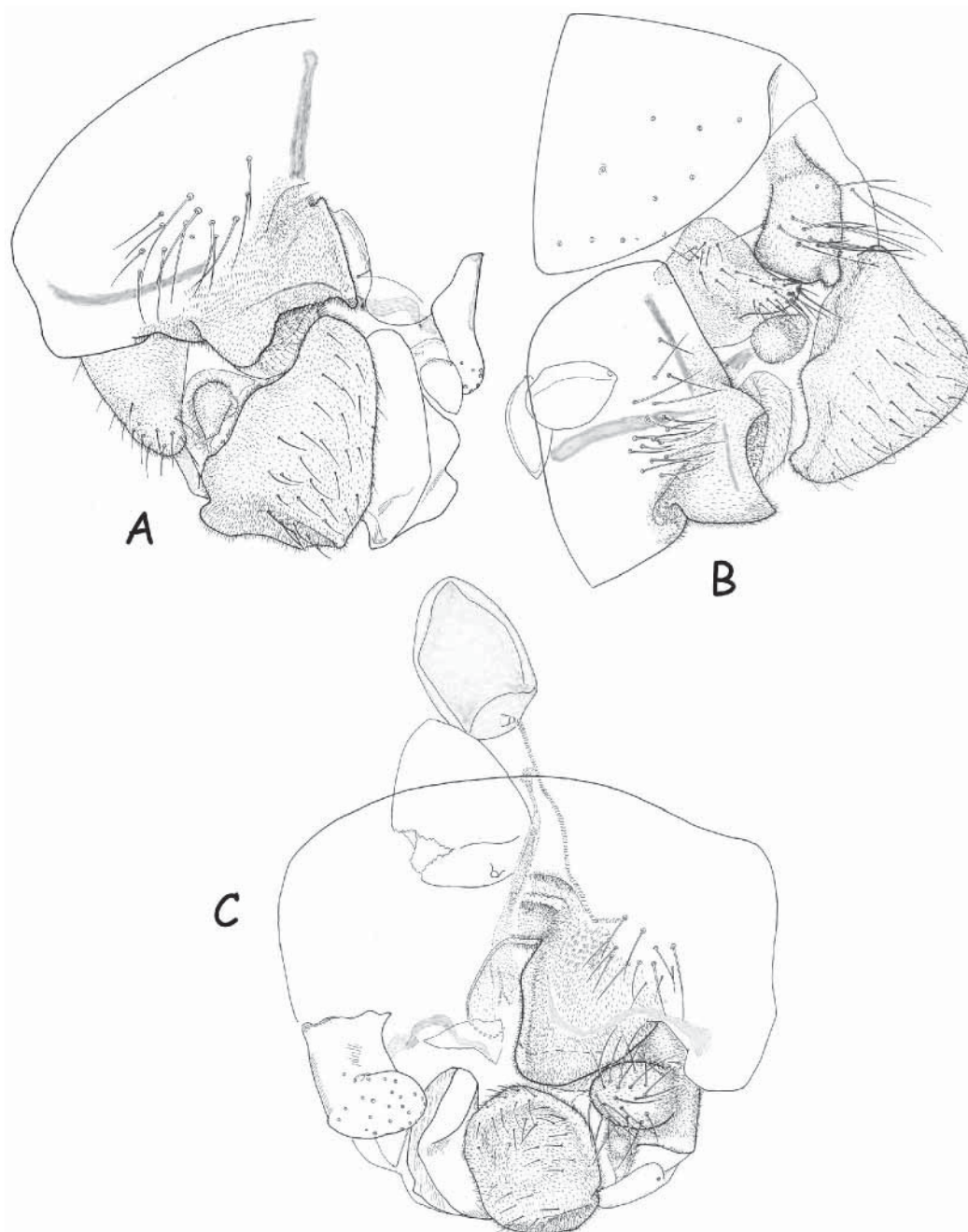
Wing: Length 3.14–3.19 mm. VR 0.85–0.89. Punctuation of membrane visible with 125x magnification. Costa moderately produced beyond  $R_{4+5}$ . R with 12 setae.  $R_1$  with 13 or 14 setae and 2 sensillae campaniformia.  $R_{2+3}$  with 2 sensillae campaniformia.  $R_{4+5}$  with 10 or 11 setae and 4–6 sensillae campaniformia. Alula with 7 or 8 setae. Squama with marginal fringe of about 32–35 setae.

Legs: Comb of hind tibia with 13 setae. Tarsi 1–3 respectively with following numbers of apical/preapical pseudospurs: 2/0, 2/0, 0/0 on  $p_1$ ; 2/10, 2/2, 0/0 on  $p_2$ ; 2/14, 2/4, 0/0 on  $p_3$ . Hind leg with dense sensilla chaetica distributed from 0.08 to 0.63 on in  $ta_1$ .  $Ta_4$  cordiform.

**TABLE 5.** Lengths (in  $\mu\text{m}$ ) and proportions of legs in female *Diamesa* sp. *Himalaya1* ( $n = 1$ ).

	fe	ti	$ta_1$	$ta_2$	$ta_3$	$ta_4$	$ta_5$	LR	BV	SV
$p_1$	1087	1299	874	407	351	95	133	0.67	3.31	2.73
$p_2$	1181	1205	555	303	189	104	114	0.46	4.14	4.30
$p_3$	1323	1488	898	502	275	114	133	0.60	3.63	3.13

Genitalia (Figs. 3A–B): Sternite VIII with 16–20 setae on each side. Gonocoxapodeme fairly distinct, with caudally displaced separate part running near posterior margin of gonapophysis. Gonapophysis VIII with flap composed of 2 parts, a mesal part with acute projection, and a lateral tonguelike lobe separated by rugulose cavity near base of ventrolateral lobe. Seminal capsules with short, sclerotised neck, capsule surface with weak granulation. Seminal ducts relatively short. Gonocoxite IX with 26–28 setae distributed from base to apex over longitudinal ridge; projection at apex relatively weak. Tergite IX deeply divided into 2 plates, each with 11 or 12 setae and a knoblike projection posterolaterally. Segment X with prominent protrusion directed slightly anterolaterad. Cerci somewhat boot-shaped in lateral view.



**FIGURES 3A–C.** *Diamesa* spp., female genitalia. A–B, *Diamesa* sp. Himalaya1 in ventral and lateral view. C, *Diamesa* sp. Himalaya2 in ventral view.

*Remarks:* The female genitalia of this species are unique but the specimens cannot at present be associated with any males of *Diamesa*. I refrain from describing it as a new species because there are several possible candidates of Himalayan *Diamesa* with unknown females. The concave margin of the gonapophysis is particularly distinctive. It

makes the flap appear more or less with two pointed lobes. The somewhat rugulose area between these lobes extends to a cavity beneath the flap and may fit the ventral projection of the cerci during oviposition. The characteristic shape of the cerci seems similar to a species described (Tokunaga 1966:fig.7b) from Afghanistan as *Diamesa* sp. near *confluens* Kieffer.

*Material studied*: China: Tibet, Rongbuk, 7.Jul. 1993, leg. T. Solhøy, det. E. Willassen, EW70, 1 female; China: Tibet, Rongbuk, water trap, 8–10.7 1993, leg. T. Solhøy, det. E. Willassen, EW96 (IZAS).

*Genetics*: Genbank accession numbers AM051227–28, mitochondrial COII gene for cytochrome oxidase subunit II.

### ***Diamesa* sp. Himalaya2**

(Fig. 3C)

#### *Female imago* ( $n = 1$ )

Head: Antenna with ultimate flagellomeres lost. Pedicel with 1 seta. Dorsal sensilla coeloconica on flagellomeres 1–3. Coronal suture complete. Number of temporal setae 34, including 7 postorbitals; frontals and orbitals slightly separated from remaining verticals. Eyes hairy, fairly small and moderately projecting dorsomesally. Clypeus 130  $\mu\text{m}$  long, 170  $\mu\text{m}$  wide, without setae. Palps short; lengths of palpomeres ( $\mu\text{m}$ ): 33, 55, 115, 72, 77. Second palpomere with or without distal campaniform sensilla. Third palpomere with sensory pit. Tentorium tube-like without anterolateral projection.

Thorax: Antepronotum with 11 lateral setae. Acrostichals absent. Dorsocentral setae, 10, in 1 row, 11 prealars behind callus, 36 scutellars. Protuberance of epimeron II with 14 setae.

Wing: Length 4.49 mm. VR 0.94. Punctuation of membrane visible with 125x magnification. Costa moderately produced beyond  $R_{4+5}$ . R with 15 setae.  $R_1$  with 12 setae and 3 sensillae campaniformia.  $R_{2+3}$  with 1 sensillae campaniformia.  $R_{4+5}$  with 11 setae and 2 sensillae campaniformia. Alula without setae. Squama with marginal fringe of about 45 setae.

Legs: Comb of hind tibia with 19 setae. Tarsi 1–3 respectively with following numbers of apical/preapical pseudospurs: 2/8, 2/2, 2/1 on  $p_1$ ; 2/21, 2/19, -/- on  $p_2$ ; 2/25, 2/10, 2/2 on  $p_3$ . Hind leg with about 170 sensilla chaetica distributed from 0.14 to 0.95 on in  $ta_1$ .  $Ta_4$  cordiform.

**TABLE 6.** Lengths (in  $\mu\text{m}$ ) and proportions of legs in female *Diamesa* sp. *Himalaya2*.

	fe	ti	$ta_1$	$ta_2$	$ta_3$	$ta_4$	$ta_5$	LR	BV	SV
$p_1$	1630	1748	1087	483	313	123	189	0.62	4.03	3.11
$p_2$	1890	1772	851	407	-	-	-	0.48	-	4.31
$p_3$	2008	2055	1158	644	455	133	189	0.56	3.67	3.51

Genitalia (Fig. 3C): Sternite VIII with 12 or 13 setae on each side; caudolateral margin with deep incision near gonocoxite IX. Gonocoxapodeme distinct and running almost to anterior margin of genital chamber. Gonapophysis VIII with caudomesally projecting flap covering most of ventrolateral lobe. Seminal capsules large, ovoid, with short neck; capsule surface with weak granulation. Seminal ducts relatively long. Gonocoxite IX with massive rounded projection bearing close to 30 long setae. Tergite IX completely divided into 2 narrow plates, each with 10 setae. Segment X with small, ventrally directed protrusion. Cerci more or less oval in lateral view.

*Remarks:* The short palps, the relatively small clypeus which is devoid of setae, and the massive projections of the gonocoxites are distinctive features of this species. Sensilla chaetica distributed nearly to the distal end of  $ta_1$  is also unusual in *Diamesa*. Spermathecae of similar size have previously been observed only in the *Diamesa dampfi* group (Willassen & Serra-Tosio 1988). Characteristics of the head, including the tubelike tentorium, indicate that the male of *Diamesa* sp.2 has short antennae. I know of no likely candidates among described species and suggest that males of this species remain undiscovered.

*Material studied:* China: Tibet, Rongbuk, 5000 m asl., 8–10.7.93, leg T.Solhøy, det. E.Willassen, EW216 (IZAS).

*Genetics:* Genbank accession number AM051229, mitochondrial COII gene for cytochrome oxidase subunit II.

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## References

- Ekrem, T. & Willassen, E. (2004) Exploring Tanytarsini relationships (Diptera: Chironomidae) using mitochondrial COII gene sequences. *Insect Systematics & Evolution*, 35, 263–276.
- Flory, E.A. & Milner, A.M. (2000) Macroinvertebrate community succession in Wolf Point Creek, Glacier Bay National Park, Alaska. *Freshwater Biology*, 44, 465–480.
- Gibbs, R.A. & Cockerill, M. (1995) Working on the assembly line. Sequencer for the Macintosh (Version 3.0, March, 1995). *Trends in Biochemical Sciences*, 20, 162–163.
- Hebert, P.D. N., Ratnasingham, S., & deWaard, J.R. (2003) Barcoding animal life: cytochrome c oxidase subunit 1 divergences among closely related species. *Proceedings of the Royal Society of London Series B-Biological Sciences*, 270, S96–S99.



- Kaul, B.K. (1970) Torrenicole insects of the Himalaya: II. Two new Diamesini (Diptera, Chironomidae) from the northwest Himalaya. *Oriental Insects*, 4, 293–297.
- Koshima, S. (1984) A novel cold-tolerant insect found in a Himalayan glacier. *Nature*, 310, 225–227.
- Lods-Crozet, B., Lencioni, V., Olafsson, J.S., Snook, D.L., Velle, G., Brittain, J.E., Castella, E., & Rossaro, B. (2001) Chironomid (Diptera : Chironomidae) communities in six European glacier-fed streams. *Freshwater Biology*, 46, 1791–1809.
- Makarchenko, E.A. (1989) Novyi i maloizvestiyi vidy roda *Diamesa* (Diptera, Chironomidae) iz Tadzhikistana. *Vestnik Zoologii*, 2, 82–85.
- Makarchenko, E.A. & Bulgakov, G.P. (1986) Novyi vid *Diamesa* Mg. (Diptera, Chironomidae) iz Uzbekistana. *Biologiya vnutrennikh vod*, 70, 37–42.
- Makarchenko, E.A. & Kobayashi, T. (1997) *Diamesa amanoi* sp. n., a new species of Diamesinae (Diptera, Chironomidae) from Nepal, with notes on taxonomy and distribution of some *Diamesa* Meigen. *Medical Entomology and Zoology*, 48, 45–48.
- Reiss, F. (1968) Neue Chironomiden-Arten (Diptera) aus Nepal. *Khumbu Himal*, 3, 55–73.
- Sæther, O.A. (1968) Chironomids from the Finse area, Norway, with special reference to their distribution in a glacier brook. *Archiv für Hydrobiologie*, 64, 426–483.
- Sæther, O.A. (1980) Glossary of chironomid morphology terminology (Chironomidae: Diptera). *Entomologica Scandinavica Supplement*, 14, 1–51.
- Sæther, O.A. & Willassen, E. (1987) Four new species of *Diamesa* Meigen, 1835 (Diptera, Chironomidae) from the glaciers of Nepal. *Entomologica Scandinavica Supplement*, 29, 189–203.
- Serra-Tosio, B. (1983) Nouveaux Diamesinae de la Paléarctide méridionale et orientale (Diptera, Chironomidae). *Spixiana*, 6, 1–26.
- Shilova, A. (1978) Novaya nakhodka *Diamesa lavillei* (Diptera, Chironomidae) v faune Sovetskogo Soyuza. *Zoologicheskii Zhurnal*, 57, 142–143.
- Singh, S. (1958) Entomological survey of the Himalaya. Part XXIX. On a collection of nival Chironomidae (Diptera) from the north-west Himalaya. *Proceedings of the Indian National Science Academy*, 28 (Sec.B), 308–314.
- Steffan, A.W. (1971) Chironomid biocoenoses in Scandinavian glacier brooks. *Canadian Entomologist*, 103, 477–486.
- Steffan, A.W. (1974) Die Lebensgemeinschaft der Gletscherbach-zuckmücken (Diptera-Chironomidae) – eine Extrembiozönose. *Entomologisk Tidskrift, Supplement*, 95, 225–232.
- Sunnucks, P. & Hales, D.F. (1996) Numerous transposed sequences of mitochondrial cytochrome oxidase I-II in aphids of the genus *Sitobion* (Hemiptera: Aphididae). *Molecular Biology and Evolution*, 13, 510–524.
- Swofford, D.L. (2002) *PAUP\*. Phylogenetic analysis using parsimony (\*and other methods). Version 4*. Sinauer Associates, Sunderland, Massachusetts
- Tokunaga, M. (1966) Some nematoceros Diptera of the northeast of Afghanistan. *Results of Kyoto University Scientific Expedition to Karakorum and Hindukush*, 1955, 273–286.
- Willassen, E. & Serra-Tosio, B. (1988) Description de trois femelles de *Diamesa* Meigen (Diptera, Chironomidae), dont *D. cinerella* Meigen (lectotype et paralectotype). *Spixiana Supplement*, 14, 91–100.